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Existence and Functions of a Gel Filled Primary Body Cavity in Development of Echinoderms and Hemichordates

RICHARD R. STRATHMANN

Friday Harbor Laboratories and Department of Zoology, University of Washington, 620 University Road, Friday Harbor, Washington 98250

Abstract. An extensive gelatinous material occupies the primary body cavity of larval echinoderms (auricularia, bipinnaria, ophiopluteus, and echinopluteus) and hemichordates (tornaria). Its presence and its recovery of shape following application and release of force were demonstrated by dissection of larvae in a suspension of sumi ink. A gel in the primary body cavity explains structures that occur in all of these five larval forms: (1) concave body surfaces bounded by thin epithelia and (2) muscles unopposed by other muscles. A gel filled primary body cavity invalidates deductions of morphogenetic mechanisms that assume a fluid filled cavity, an assumption implicit in many models of blastulation, gastrulation, and movement of mesenchyme cells. A gelatinous primary body cavity permits body plans and morphogenetic processes not possible with a fluid filled cavity and permits development of large larvae with little cellular material. The taxonomic distribution of gel filled body cavities is not known, but gel filled cavities are possible wherever fluid motion has not been demonstrated or is not a functional necessity.

Introduction

Many small animals lack both a muscular body wall and any apparent skeletal support, yet they maintain and alter elaborate body shapes. The embryos and larvae of echinoderms and hemichordates are examples of such animals, and the means by which they maintain their shapes are examined here. The means are not obvious. The body wall in these larvae is a thin epidermis. Some of these larvae (echinoplutei, ophioplutei) have a skeleton of calcite rods that support projecting arms (Fig. 1D), but others (tornaria, auricularia, bipinnaria) do not (Figs. 1ABC, 2AD). Even in the larvae with calcite rods, the epidermis is not everywhere stretched on the skeletal framework. In all of these larvae the thin sheet of epidermal cells appears inadequate to support itself. Two hypotheses for support of the epidermis have been stated or implied throughout an extensive literature on these larval forms. The most common assumption has been that the primary body cavity is filled with fluid and the thin body wall presumably supported by higher internal pressure (a hydrostatic skeleton), but no evidence for a hydrostatic skeleton has been presented. The other hypothesis is that the primary body cavity contains gelatinous supporting material (Ruppert and Balser, 1986; Summers *et al.*, 1987), perhaps like the mesogloea of jellyfish. Material that might have a supporting role has been demonstrated in the blastocoel of blastulae and gastrulae of echinoderms (Monné and Slautterback, 1950; Endo and Noda, 1977; Katow and Solursh, 1979; Kawabe *et al.*, 1981; Abed and Crawford, 1986; Summers *et al.*, 1987). However, these studies employed histological methods or electron microscopy. None of these studies tested the fluid or gelatinous character of the contents of the primary body cavity; and gelatinous material has not been obtained from live specimens. Thus, even a qualitative indication of the mechanical properties of material in the primary body cavity has been lacking.

Gelatinous material in the primary body cavity is here demonstrated to occur in all five types of feeding larvae of echinoderms and hemichordates, even in those with calcite skeletal rods. Available evidence therefore points to gelatinous support of these larval bodies. Support by a gel in the primary body cavity explains many features of shape, musculature, movement, and morphogenesis of these larvae.

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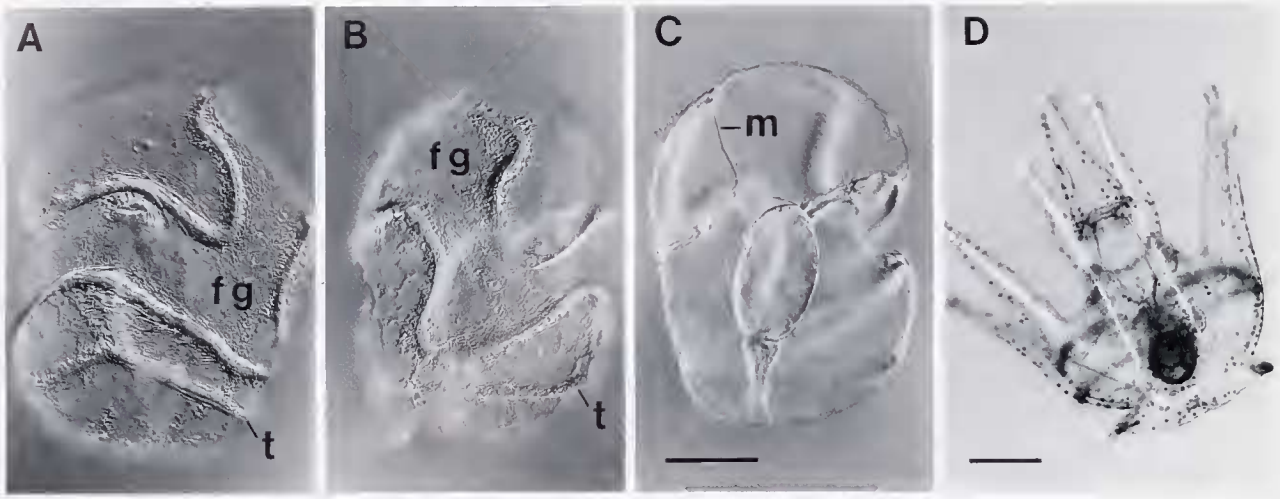

Figure 1. (A, B, C) Tornaria of *Ptychodera flava* with a concave to flat food groove (fg) upstream from the ciliated band. Body surfaces downstream from the band are flat to convex. Downstream areas include a separate locomotory band, the telotroch (t). The optical sagital section in C shows the thin epidermis, spaceous primary body cavity, and the muscle (m) between coelom and apical organ. Differential interference contrast optics. D: Echinopluteus of *Strongylocentrotus franciscanus* with skeletal rods in all arms and a less spaceous primary body cavity. Some skeletal rods are brightly illuminated because of partially crossed polarizing filters. Scale lines are 200 μm.

Materials and Methods

Species are listed in Table I. Echinoderm larvae were reared at the Friday Harbor Laboratories from fertilized eggs in large (2 l) cultures (Strathmann, 1987). Ova and sperm of the hemichordate were obtained by leaving ripe adult worms in sand in still water at air temperature with occasional water changes until they spawned (Hadfield, 1975, and pers. comm.). After fertilization the hemichordate embryos and larvae were reared at the Kewalo Marine Laboratory in beakers with an airlift circulation (Miller and Hadfield, 1986). A single and unidentified large auricularia with wheel ossicles, obtained from the plankton near Honolulu, was also tested.

Live larvae were torn into two or more fragments with tungsten needles that were sharpened in molten $NaNO_2$ (T. E. Schroeder, pers. comm.). The larvae were dissected and observed in a suspension of particles of sumi ink ground on an ink stone (Schroeder, 1980a, b). The ink particles served several purposes. Because the ground substance of the primary body cavity itself was not visible with bright field or differential interference contrast (DIC) optics, the ink particles were needed to mark the boundaries of gelatinous material by decorating its surface and by exclusion of the ink suspension. The ink particles indicated motion of fluid, and the ink suspension mixed with body fluids. Because cells out of focus can resemble homogeneous and transparent gelatinous material, the ink particles were also useful as a marker for surfaces in focus.

Most larvae were dissected in seawater. Some specimens were dissected in isosmotic $MgSO_4$ or $MgCl_2$, which relaxed the larvae and may have aided removal of epidermis. However, because changes in concentration of calcium and other ions affect the viscosity of connective tissues of echinoderms (Motokawa, 1984; Wilkie, 1984), replacement of seawater with isosmotic $MgSO_4$ or $MgCl_2$ may have affected viscosity of materials in the primary body cavity. Except where isosmotic $MgSO_4$ or $MgCl_2$ is specified, seawater was the solution used in the results reported here.

I examined the fragments under a coverglass that was supported at its corners by pieces of plasticene (Clayola®). The test for gel was conservative in that gel was recorded only if a number of criteria were met. More than half the dissections demonstrated gel, and it is likely that gel was present even when these criteria were not met. Clear material from larval fragments was judged to be fluid if it sheared and eventually mixed with the ink suspension when the coverglass was depressed vertically and slid horizontally so as to compress or shear the specimen. Clear material unbounded by epithelia or membranous sheets was judged to be gelatinous if it did not mix with the ink suspension and if it returned to its original shape and position even when the coverglass was not returned to its original position. (Because of low Reynolds numbers, return of fluid to its original position after reversal of motion of the coverglass was expected and disregarded in these tests.) The presence or absence of bounding epithelia or other membranes was determined by observation under a 40× objective with DIC optics.

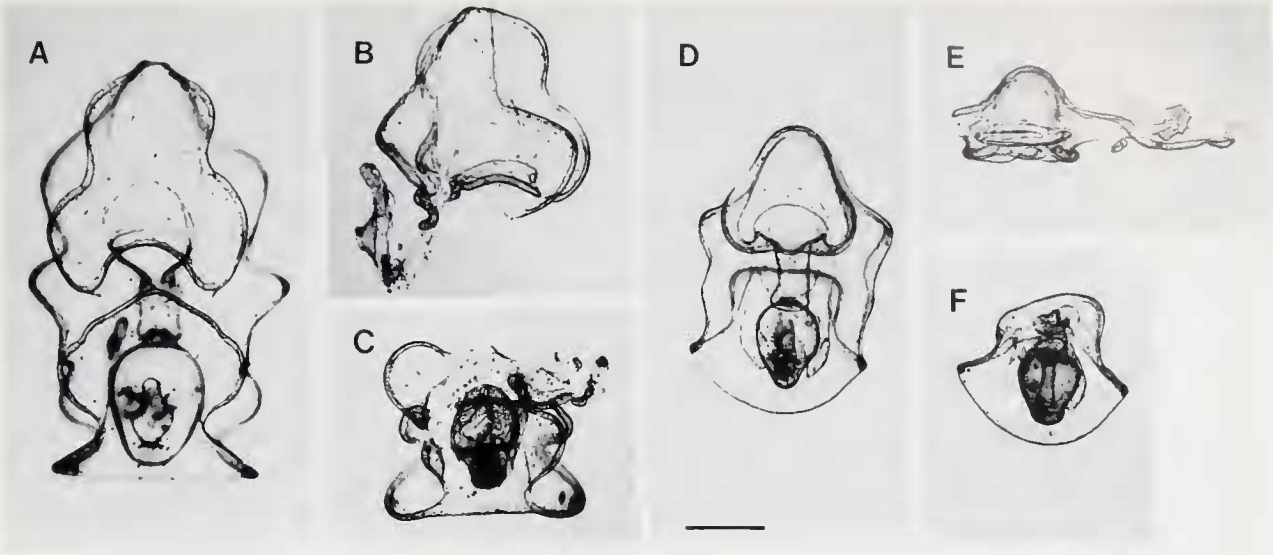

Figure 2. (A) Intact auricularia of *Parastichopus californicus* and anterior (B) and posterior (C) halves in seawater. (D) Intact bipinnaria of *Asterina miniata* with anterior (E) and posterior (F) halves of the same larva in isosmotic $MgCl_2$. The larvae were torn apart with needles but the halves retain their shape. Scale line is 200 μm for all figures.

When positive tests for gelatinous material were initially doubtful, tests were repeated until several specimens gave clear results. In addition, movement of ink, fluid, and gel was videorecorded for the tornaria so that impressions could be verified or rejected by repeated replay of events. Demonstration of the gelatinous material was not possible for every individual larva because ink adhered to the gelatinous material, accumulating on its surface and hiding it. Also, observation of gelatinous material depended on optical sections through a free surface, but orientation of larval fragments suggested that gelatinous material adhered to the glass slide and coverglass, thereby reducing opportunities to view torn edges of gel projecting into the ink suspension.

Results

Larvae of all five classes (Table I) had gelatinous material in their primary body cavities. The primary criteria for this conclusion were that the material excluded a suspension of ink particles during repeated compression and shear, did not shear like the surrounding fluid, returned to its original shape when deformed, and was not bounded by a membrane visible with a 40× objective and differential interference contrast (DIC) optics. These criteria were met by material from torn fragments of all five larval forms. The species tested in this way were *Ptychodera flava, Parastichopus californicus,* an unidentified large auricularia with wheel ossicles, *Asterina miniata, Ophiopholis aculeata,* and *Strongylocentrotus franciscanus.* All were dissected live, and all were in seawater, except for *A. miniata* and *O. aculeata,* which were in isosmotic $MgSO_4$. Ink particles were in brownian motion immediately adjacent to the surface of the gelatinous material. The boundary was distinct and abrupt. The surface of the gel in torn fragments was often irregular, as in Figure 3ABCD, not smooth as would be expected of an immiscible fluid or fluid bounded by a thin membrane. In most cases the material was visible at the edge of a torn fragment of the larva, as in Figure 3ABCEF. In a few cases (*P. flava, P. californicus, A. miniata, S. franciscanus*) I obtained isolated fragments of gel with only a few cells attached, as in Figure 3D.

In all five types of larvae the gel appeared transparent and uniform when viewed with a 40× objective and DIC optics (Figs. 3EF, and 4AB). The gel was not visibly granular, membranous, or fibrous, unlike cells, cell debris, and some other materials associated with cut fragments.

Some observations suggested that the gel filled most or all of the primary body cavity. In one dissection the cut

Table I

Larvae tested for gel in the primary body cavity

Phylum	Class	Species	Larval form
Hemichordata	Enteropneusta	*Ptychodera flava*	Tornaria
Echinodermata	Holothuroidea	*Parastichopus californicus*	Auricularia
	Asteroidea	*Asterina miniata*	Bipinnaria
	Ophiuroidea	*Ophiopholis aculeata*	Ophiopluteus
	Echinoidea	*Strongylocentrotus franciscanus*	Echinopluteus

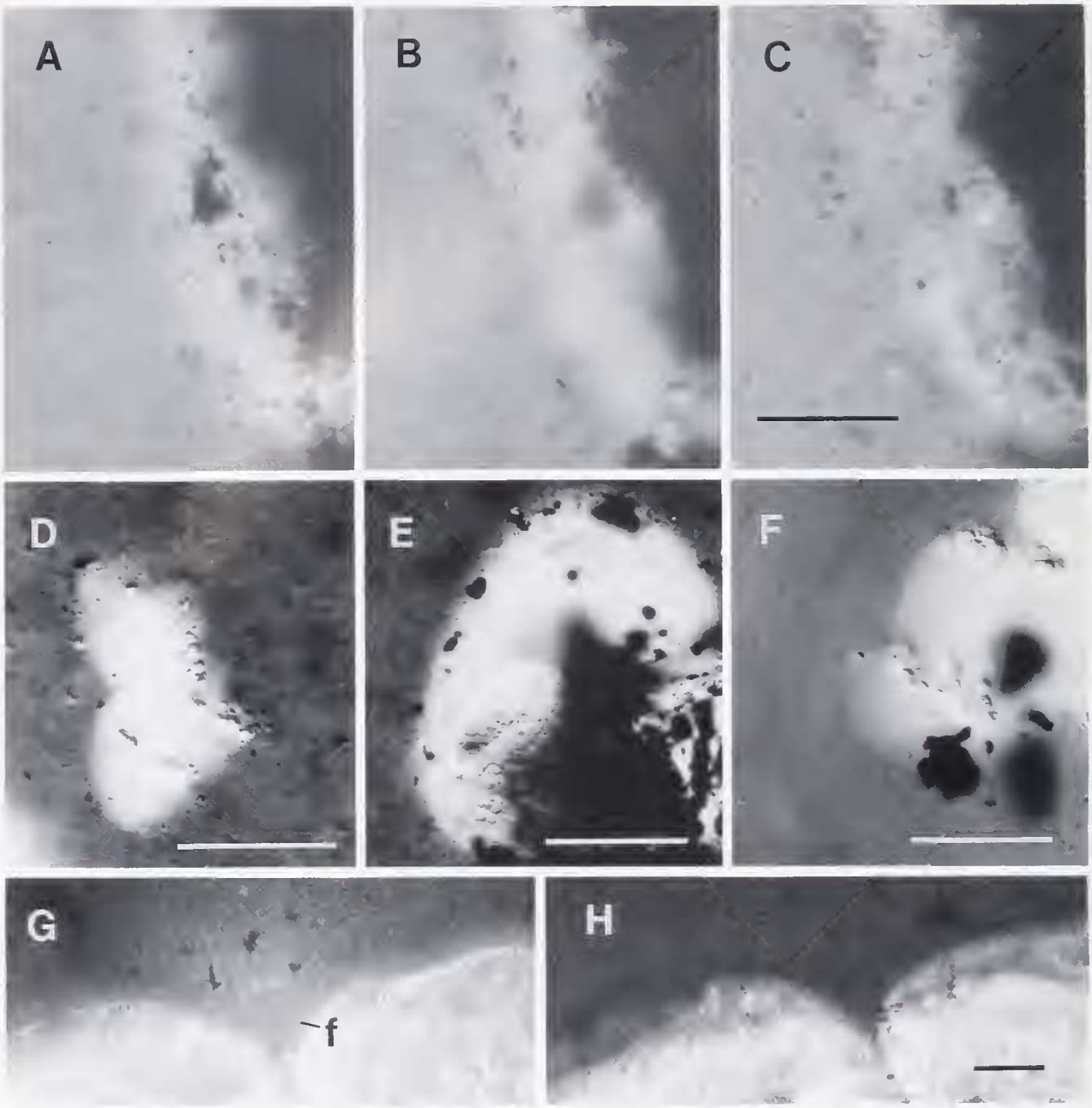

Figure 3. (A, B, C) High, middle, and low focus optical sections through gelatinous material at the cut edge of a tornaria of *Ptychodera flava.* Ink particles suspended in seawater show boundaries of the gelatinous material. (D) An isolated fragment of gelatinous material from cut fragments of the auricularia of *Parastichopus californicus.* The material is slightly compressed by the coverglass, surrounded by ink particles suspended in seawater, and partially bounded by ciliated cells on the right side. (E, F) Gelatinous material from pieces of the bipinnaria of *Asterina miniata* surrounded by ink particles suspended in isosmotic $MgSO_4$. Both pieces are strongly compressed by the coverglass. In E the gelatinous material surrounds part of the larval gut. In F the ink particles are in motion past the gelatinous material. (G, H) Piece of the tornaria of *P. flava* strongly compressed by the coverglass and surrounded by ink particles suspended in seawater. A clear fluid (f) has been extruded from the larval body in G but has been dispersed by motion of the coverglass in H. All scale lines are 50 μm.

half of a tornaria (*P. flava*) adhered to the coverglass. Pockets of ink suspension were trapped between this larval half and the coverglass, but excluded from the spacious primary body cavity to the side and below. The pockets of ink suspension were irregular and not bounded by a visible membrane. The ink particles were

Figure 4. (A, B) Arms of the ophiopluteus of *Ophiopholis aculeata* with most of the epidermis stripped away; ink particles are suspended in isosmotic $MgSO_4$. A blob of gelatinous material is almost detached in B. Scale lines are 50 μm. (C) Arm of the echinopluteus of *Strongylocentrotus franciscanus* with most of the epidermis stripped away; ink particles are in seawater. C is at lower magnification than A and B. All specimens are strongly compressed by the coverglass.

in brownian motion immediately adjacent to the invisible surface of the bounding gel. I therefore concluded that the irregular pockets were the torn surface of a gel that filled the body cavity.

Gelatinous material also extended into arms of plutei from which the epidermis had been stripped (*O. aculeata* in isosmotic $MgSO_4$, *S. franciscanus* in seawater; Fig. 4). In one dissection of *S. franciscanus* an isolated blob of gel remained skewered on an arm rod like a kebab. Thus even body parts with skeletal support also had gelatinous support. The posterior part of the bodies of the ophiopluteus and echinopluteus also contained gelatinous material (same two species).

The gel partially or completely surrounded the gut (Fig. 3E) and esophagus of fragments of the bipinnaria of *A. miniata* (dissected in isosmotic $MgSO_4$).

There was also evidence against the alternative hypothesis that hydrostatic pressure maintains the larval shape. When tornariae (*P. flava*) and auriculariae (*P. californicus* and large larva with wheel ossicles) were torn in half in seawater, the thin epidermis did not collapse like a punctured balloon. The same result was obtained with the bipinnaria of *A. miniata* in both isosmotic $MgCl_2$ and seawater. Fragments of all three larval forms maintained the form that they had in the intact larva (as in Fig. 2 for auricularia and bipinnaria). Also, anterior and posterior halves of auriculariae (*P. californicus*) and bipinnariae (*A. miniata*) were distorted by compression under the coverglass but returned to their original shape when the coverglass was raised. Cut edges of the halves were not visibly sealed by epidermis; the primary body cavity appeared to remain open. Ink particles entered the oral cavity of the larval fragments but not the primary body cavity at the cut edges.

Fluid as well as gel was extruded from fragments of larvae under compression (Fig. 3GH). It is possible that parts of the primary body cavity were fluid but also possible that the fluid came from the gut or coelom or that some gel became fluid as a result of the dissection. I could not quantify comparisons, but it appeared that more fluid was extruded from the bipinnaria of *A. miniata* than from the auricularia of *P. californicus* when both were in seawater, and it was more difficult to demonstrate gel in the body cavity of *A. miniata* than of *P. californicus*.

The apparent volume of gelatinous material decreased when larval fragments were repeatedly compressed under a coverglass. The gelatinous material may have disappeared because it coated the glass, was coated with ink particles, or eventually became fluid when freed of the surrounding epithelia.

Discussion

Observations on living material demonstrate a gelatinous material in the primary body cavity of larval echinoderms and hemichordates. Support by gelatinous material in the primary body cavity explains many aspects of shape and musculature of these larvae and has implications for their development, as discussed below.

A gel can be any shape, and a gel filled body cavity may permit close approximations to optimal shapes. Placement of ciliary bands on ridges permits more effective propulsion of water (Emlet, 1985) and capture of particles (Strathmann, 1975). Depressed food grooves may aid retention of transported particles. In echinoderm and hemichordate larvae, ciliary bands are on ridges and much of the area for transport of food (upstream from the ciliated band) is concave (Figs. 1ABC, 2AD). These concave regions are bounded by a very thin and flexible epidermis. Such a shape is inconsistent with the inflated appearance expected with a hydrostatic skeleton. An inflated sac (with a hydrostatic skeleton) would either impose a smoother surface topography or require

many additional internal cables or structures in the body wall to maintain the body's shape. The gel in the primary body cavity may also support the lobes or tentacles of some bipinnariae, auriculariae, and tornariae. Even some plutei, as in cidaroid echinoids (Emlet, 1988), have pronounced lobes that lack support from skeletal rods or other visible structures.

Support from gelatinous material permits development of large larvae with relatively little cellular material. The larval body is primarily a feeding machine, whose maximum capacity for clearing particles from suspension depends on the length of the ciliated band (Strathmann, 1971; M. Hart, pers. comm.). Strong positive allometry in ciliated band relative to body length and near isometry in band length to body protein and band length to metabolic capacity has been determined for echinoid larvae (McEdward, 1984, 1986). A strong positive allometry in band length to body length has been estimated for hemichordate larvae (Strathmann and Bonar, 1976). Except for the ciliated band, the body wall consists of an extremely thin sheet of epidermal cells. The body wall need not and does not provide much mechanical support for the ciliated band.

There is also an economy in muscles used for ingestion (circular esophageal muscles), rejection of particles (oral dilators of plutei and dorsal contractors of bipinnariae), and change of direction of swimming (dorsal contractors of bipinnariae) (Strathmann, 1971). None of these muscles is opposed by another muscle, yet the body rebounds to its resting shape when these muscles relax. The muscle that depresses the apex of the tornaria (Fig. 1C) also consists of a thin strand with no opposing muscle. For all of these activities, the gelatinous material in the primary body cavity is the only known elastic element that could oppose the muscles. Opposing muscles are unnecessary, and operation of muscles imposes few constraints on larval shape. Maintaining and changing shape requires very little cellular material in the body wall.

A gelatinous supporting skeleton also has implications for morphogenesis. The epithelia around the primary body cavity are not surrounding a fluid. An extracellular network of organic material in the primary body cavity of echinoderm embryos has been indicated by histological preparations and electron microscopy of preserved blastulae and gastrulae (Monné and Slautterback, 1950; Endo and Noda, 1977; Katow and Solursh, 1979; Kawabe *et al.*, 1981; Abed and Crawford, 1986). The extent of this material was difficult to ascertain because of shrinkage from fixation and preparation before observation. Berg and Akin (1971) collected fluid from echinoid blastulae by centrifugation. More recently Summers *et al.* (1987) demonstrated an extensive and oriented blastocoelic organic matrix in echinoid blastulae and gastrulae prepared by freeze-substitution. My observations with sumi ink extend those of Summers *et al.* (1987) by giving a direct indication of the mechanical properties of gelatinous material from body cavities of larvae. The observations also extend evidence of such material to additional classes of echinoderms and to hemichordates.

These observations on gel filled primary body cavities invalidate a critical assumption underlying a number of morphogenetic models that concern changes in shape of sheets of cells or movements of mesenchyme. A common practice in studies of the mechanics of morphogenesis from Gustafson and Wolpert (1962, 1963) to the present has been to deduce forces operating on sheets of cells from changes in the shape of tissues, but such models have implicitly assumed that blastocoels or other body cavities were fluid filled. This assumption is false for the primary body cavity in larval echinoderms and hemichordates and could be false for any body cavity in which fluid motion has not been observed. Also, mesenchyme cells are not traversing a fluid filled space in these larvae. The observations of morphogenetic movements remain sound, but interpretations of forces must be reexamined.

The role in morphogenesis of fibrillar material observed on the blastocoel wall in fixed blastulae and gastrulae may also need reinterpretation. Some of this material may be condensed gel.

A gel filled cavity could permit morphogenetic processes not possible in a fluid filled cavity. If epithelial or mesenchyme cells can dissolve and reform the gel, the gel filled cavity should permit greater freedom in generating forms and distributing cells than would a fluid filled cavity. The viscosity of connective tissue of adult echinoderms is under nervous control (Motokawa, 1984; Wilkie, 1984), and alteration of viscosity in the primary body cavity is a possibility that should be explored. Also, the mesenchyme cells in the primary body cavity of echinoderms and hemichordates are supported by a gelatinous material, and their movements may include complete detachment from other cells.

Gel filled cavities could play a major role in other embryos, larvae, and small adults. The plutei demonstrate that an internal mineral skeleton does not preclude the existence and usefulness of a gel filled body cavity. The taxonomic distribution of gelatinous support cannot be deduced from the presence or absence of other supporting structures.

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